Detailed materials and methods

Plasmid constructs. The *ORCA3* promoter (accession number AJ251250) was isolated by inverse PCR with primers OR5 (5´-AGATCTCATATGTCCGAAGAAACTATTTCCGTCTCAG-3') and OR8 (5'-GATGAATAGAGTGAGGAGTGG-3') on EcoRI-digested and re-ligated genomic DNA. The *ORCA3* promoter fragment generated on genomic DNA by PCR was cloned into pGEM-T Easy (Promega) such that the OR8 sequence flanked the SP6 side of the polylinker. An EcoRI fragment containing the *ORCA3* promoter from positions -826 to -53 relative to the ATG codon (AJ251250) was transferred to GusXX (Pasquali et al., 1994) to generate the plasmid Δ826GusXX. *ORCA3* 5' promoter deletion fragments Δ606, Δ190 and, Δ88 were generated by PCR on plasmid Δ826GusXX using primers Δ606 (5´-CGTCTAGAAAATACACTATCTAAACAT-3´), Δ190F (5´-AACTGCAGTAATTGCACCTCCCAAGCGC-3') and Δ88 (5-AACTGCAGCTTAGTATATAAATTCCACTC-3'), respectively, combined with the GUS3 primer (5'-CTGAATGCCCACAGGCCGTCGAG-3'). The PCR fragments were cut with XbaI/EcoRI, PstI/EcoRI and PstI/EcoRI, respectively, and cloned into GusXX. *ORCA3* 5' promoter deletion fragments Δ354, Δ264 and Δ121 were isolated by digestion of the Δ826 *ORCA3* promoter with DraI/EcoRI, SnaBI/EcoRI and SspI/EcoRI, respectively, and the appropriate fragments were cloned into GusXX digested with SmaI/EcoRI. *ORCA3* internal promoter deletion 264Δ1 was generated by PCR with primers Δ190R (5´-GAAGATCTGTCACGTTTACACAGAATTAA-3´) and T3 on plasmid Δ264-

GusXX. The PCR product was cut with BglII/BamHI/SacI, and a 75 bp BamHI/BglII fragment was cloned into Δ121GusXX digested with BamHI. To generate 264Δ2, a 145 bp BamHI/SspI fragment was cloned into Δ88GusXX digested with BamHI/SmaI. To generate 264Δ3, a PCR product produced with primers Δ190F and GUS3 on Δ826GusXX was cut with AvaII, filled in with the Klenow fragment of DNA polymerase I and cut with EcoRI. The resulting 125 bp fragment was cloned into GusXX digested with EcoRI/SmaI generating Δ162GusXX. Fragment A was excised with BamHI/BglII from pIC19H-A (see below) and cloned into Δ162GusXX, resulting in 264Δ3GusXX. To construct 264Δ4, a 230 bp fragment obtained by cutting Δ264GusXX with SacI/EcoRI was cut with AvaII, filled in with the Klenow fragment of DNA polymerase I and cut with BamHI. The resulting 110 bp fragment was cloned into pIC-19H (Marsh et al., 1984) digested with BamHI/EcoRV. A BamHI/BglII fragment excised from this plasmid was cloned into Δ121GusXX cut with BamHI, resulting in 264Δ4GusXX. A 6Tcyt fragment was isolated from 6TcytGusSH-47 (Menke et al., 1999) with BamHI/PstI and cloned into Δ88GusXX to generate the control construct 6Tcyt-Δ88GusXX. *ORCA3* promoter fragment A was generated by PCR with primers Δ190R and T3 on plasmid Δ264GusXX. The PCR fragment was cut with BglII/BamHI/SacI, and a 75 bp BamHI/BglII fragment was cloned in pIC-19R digested with BamHI/BglII. To generate fragment B, a BamHI/SalI fragment was isolated from Δ190GusXX, cut with SspI, and the 85 bp BamHI/SspI fragment was cloned into pIC-19H digested with BamHI/EcoRV. To generate fragments C and D, SspI/DdeI and AvaII/DdeI fragments, respectively, were isolated from

Δ190GusXX, filled in with the Klenow fragment of DNA polymerase I and cloned into pIC-20H digested with EcoRV/SmaI such that the DdeI half-sites flanked the SmaI half-site. Fragments were tetramerized according to Ouwerkerk and Memelink (1997) using the enzyme combination BamHI/BglII. Tetramers 4A and 4B were cloned as BamHI/XhoI fragments into GusSH-47 (Pasquali et al., 1994) digested with BamHI/SalI. Tetramers 4C, 4D and its mutant derivatives were cloned into GusSH-47 as SacI/BamHI fragments. *ORCA3* promoter-*GUS* fusions were transferred from the GusXX plasmid to the binary vector pMOG22λCAT (Menke et al., 1999) as XbaI/XhoI fragments, whereas the tetramer-GUS fusions and 6Tcyt-Δ88Gus were transferred with SacI/HindIII. For the AT-hook overexpression constructs, the inserts of clones 2D38M, 2D7 and 2D173 were excised with BamHI/BglII, BamHI/XbaI or EcoRI/XbaI and cloned in expression vector pRT101 (Töpfer et al., 1987) digested with BamHI, BamHI/XbaI or EcoRI/XbaI.

Cell transformation. *C. roseus* cell line BIX was transformed using *Agrobacterium tumefaciens* strain LBA4404 containing the ternary plasmid pBBR1MCS-5 carrying the constitutive *VirGN54D* mutant gene and *ORCA3* promoter derivatives in pMOG22λCAT as described (van der Fits et al., 2000). Cell suspension cultures were grown as described (Menke et al., 1999)

RNA extraction and Northern blot analysis. RNA extraction and Northern blot analysis were performed as described before (Menke et al., 1999). Northern blots

were probed using ^{32}P-labeled DNA probes corresponding to the full-length *ORCA3*, *GUS* and *CAT* coding regions or the complete *AT-hook* and *Rps9* (encoding ribosomal protein S9) inserts.

Yeast one-hybrid screening A monomer and a dimer of the D fragment and a tetramer of the C fragment were cloned as BamHI/Bglll fragments from pIC-20H into the BamHI site of pHIS3NX (Meijer et al., 1998). Gene fusions of 1D-, 2D- and 4C-*HIS3* were transferred as NotI/XbaI fragments to pINT1 (Meijer et al., 1998). The resulting plasmids were digested with NcoI/SacI and introduced into yeast strain Y187 (Harper et al., 1993). Recombinants were selected on YPD medium containing 200 μg/ml G418, and the occurrence of double cross-over integration events between the pINT1 derivative and the chromosomal *PDC6* locus was verified via Southern blot analysis. To construct the library from MeJA-treated cells, cDNA was synthesized with a Stratagene HybriZAP-2.1 XR kit on poly(A) RNA prepared from 2 different total RNA samples, mixed in a 1:1 ratio, isolated from cultures of cell suspension line MP183L that were treated with 50 μM MeJA for 0.5 and 2 h, respectively. The amplified lambda HybriZAP-2.1 library, consisting of 4 x 10^6 independent primary transformants, was converted to a pAD-GAL4-2.1 plasmid library according to the manufacturer's instructions.

Electrophoretic Mobility Shift Assays

The inserts from pAD2D81, pAD2D206, pAD2D21, pAD2D328, pAD2D1 and pAD2D7 were isolated with BamHI/XhoI, and cloned in pGEX-KG (Guan and

Dixon, 1991). The insert from pAD2D449 was isolated with EcoRI/XhoI, and cloned in pGEX-4T1 (GE Healthcare). The insert from pAD2D173 was amplified by PCR with the primers AD173ATG (5'-GGAATTCAAAATGGATCATTCACTACCACCTC-3') and 3AD2, digested with EcoRI/XhoI, and cloned in pGEX-4T1. The inserts of clones pACT-4C19, 4C32, 4C49 and 4C87 were cloned in pGEX-KG with SmaI/XhoI, EcoRI/HindIII, SmaI/XhoI and EcoRI/XhoI, respectively. Expression plasmids were introduced in *E. coli* strain BL21 (DE3) pLysS or its derivative Rosettagami B. D wild-type and mutant fragments were isolated from pIC-20H with XbaI/XhoI and labeled by filling in the overhangs with the Klenow fragment of DNA polymerase I and α-^{32}P-dCTP. EMSAs were performed as previously described (Menke et al., 1999).

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